

## Original Research Article

### **Phytochemical screening and evaluation of the larvicidal activities of three organic extracts derived from the leaves of *Cassia siébériana* to control *Anopheles gambiae*, the vector of malaria.**

#### **Abstract**

Senegal is one of the African countries where malaria remains a major public health problem. In the fight against mosquitoes, the vector of this disease, the significant accumulation of active ingredients in ecosystems treated with synthetic insecticides poses an increased environmental problem linked to the very high persistence time.

The aim of this study is to propose an alternative method of vector control using natural products of plant origin.

Three organic extracts (cyclohexanic, chloroformic and methanolic) from the leaves of *Cassia siébériana* were tested by contact on mosquito larvae of the genus *Anopheles gambiae*. Four doses of each extract were prepared and monitored over 72 hours.

The results obtained show a variable sensitivity of the larvae, reflected in low to very high mortality rates when moving from one extract to another. The chloroform extract was the most effective, with a mortality rate of just under 90% after 48 hours' exposure. Phytochemical screening reveals that this plant is rich in secondary metabolites such as polyphenols and alkaloids, which could explain its larvicidal activity.

**Key words:** *Cassia siébériana*, *Anopheles gambiae*, larvicidal activity, organic extracts.

#### **Introduction**

Malaria is a parasitic endemic to which 2 billion people worldwide are exposed. In 2022, there will be an estimated 247 million cases of malaria and 619,000 deaths worldwide. [1]. Most of these deaths will occur in sub-Saharan Africa, with children under five being the worst affected. Senegal is one of the African countries where malaria remains a public health problem, and despite efforts at various levels to reduce its burden, the statistics remain almost unchanged. An average of 300,000 to 350,000 confirmed cases are recorded each year.

Mosquitoes are the exclusive vectors of malaria. There are currently 3,500 species of mosquito in the world. [2]. Only around sixty species, belonging to the genus *Anopheles*, are capable of transmitting plasmodium, the parasite responsible for human malaria.

A number of resources have been made available to combat vector-borne diseases.

These measures range from building villages away from the tides to draining collections of water where mosquito larvae develop.

The advent of insecticides raised hopes of eradicating malaria from areas where the disease was endemic. Thus, on the initiative of the WHO, a vast malaria eradication program had been launched in the years since the 1950s. The active ingredients of the insecticides used in mosquito control campaigns belong to the following families: organophosphates, synthetic pyrethroids, organochlorines and synthetic carbamates. [3].

When cases of resistance appeared, all hope of eradication was dashed. Although these preparations have proved highly effective against Culicidae mosquitoes, they have a

**Commented [A1]:** the abstract is sequenced as follows: introduction, methods, results and conclusion. The method used to identify secondary metabolites should be mentioned in the methods section. I believe these are precipitation and colorimetry methods.

**Commented [A2]:** it's in French. Write sieberiana instead of siébériana. The same applies throughout the manuscript.

number of drawbacks. The significant accumulation of active ingredients in treated aquatic and terrestrial ecosystems poses a pollution problem. Furthermore, the active substances in the products used have a broad spectrum of action and do not spare non-target organisms.

In addition to all these disadvantages, there is also the major problem of the development of resistance to chemical insecticides in mosquitoes. [4]. As a result, the traditional method of chemical mosquito control is increasingly giving way to biological control.

Furthermore, a systematic examination of phytochemical discoveries listed using the NAPRALERT (Natural Products Alert database) reveals that only less than 30% of plant species have been examined in detail. Given that 65% of plant biodiversity is tropical, it is clear that a large number of molecules of plant origin remain to be discovered.

As part of this vector control program, research has been carried out in our laboratory into the toxicity of a number of native plant extracts.

Three *Cassia siebériana* leaf extracts were tested on *Anopheles gambiae* mosquito larvae.

## I. Materials and methods

### I.1. Equipment

#### I.1.1. Plant material

*Cassia siebériana* leaves were harvested in May 2022 in the THIES region located 70 km from DAKAR (SENEGAL). In order to preserve the efficacy of the active ingredients, drying is carried out immediately after harvesting, in a well-ventilated place and away from light. They are then ground using an electric grinder.

#### I.1.2. Biological material

The *Anopheles gambiae* larvae tested for toxicity came from stagnant water at the Lycée Blaise Diagne (Dakar/Senegal).

The species collected were sorted and isolated from each other to avoid interspecific interactions. Only stage 3 larvae (L<sub>3</sub>) were selected for testing.

### I.2 Methods

#### I.2.1. Extraction

To extract the active ingredients, 100g of *Cassia siebériana* leaf powder was macerated in 1L of solvent for 72 h in the dark at room temperature.

Three extraction solvents were used (cyclohexane, chloroform and methanol).

Using a rotary evaporator, all the extracts were concentrated to dryness and then stored at -18°C to prevent any deterioration.

**Commented [A3]:** Justify this paragraph and correct the parts underlined in red or yellow. Write sieberiana

#### I.2.2. Phytochemical screening

The various extracts were subjected to various phytochemical tests to identify the main chemical groups contained in them, using the standard method based on staining and precipitation reactions. Several types of reagents were used for this purpose [5].

#### Characterization of polyphenols

An infusion was prepared by dissolving 1mg in 20mL of distilled water. The mixture was left to stand for 25 min and then filtered. A 2 mL sample of the filtrate was taken and placed in test tubes. A few drops of an alcoholic solution of FeCl<sub>3</sub> were added. The

**Commented [A4]:** 20 ml

appearance of a blackish-

blue color indicates the presence of polyphenols. The same filtrate is also used to characterize flavonoids and tannins.

#### Characterization of flavonoids

First, 1 ml of the previous infusion is added, followed by 1 ml of hydrochloric acid. A few shavings of magnesium are added to this mixture. The appearance of an orange-pink color indicates the presence of flavones, a purplish-pink color indicates the presence of flavanones and a red color indicates the presence of flavanols.

#### Catechic tannins or non-hydrolysable tannins

1 mL of the infusion is added to 0.5 mL of Stiasny's reagent (a mixture of methanal and hydrochloric acid). The resulting mixture was heated to 90° C for 15 min. The appearance of a precipitate indicates the presence of catechic tannins.

#### Gallic or condensed tannins

To reveal the gallic tannins, the contents of the tubes after the catechin tannin test were first filtered, then 0.5 mL of the filtrate was saturated with sodium acetate and finally 0.2 mL of a 2% solution of FeCl<sub>3</sub> was added to this mixture. The appearance of a blue-green color indicates the presence of gallic tannins.

#### Alkaloids

1 mg of each dry extract was dissolved in 20 ml of 10% concentrated sulphuric acid. A few drops of Dragendoff's reagent were added to this mixture. The appearance of an orange-red precipitate indicates the presence of alkaloids.

### 1.2.3. Toxicity tests

The methodology of our tests is inspired by the sensitivity test technique standardized by the World Health Organization [6].

The doses were prepared by dissolving dry alcoholic extracts (such as methanolic extract), chloroform and cyclohexane in dimethyl sulphoxide (DMSO). Four doses were prepared for each extract: dose1 (50mg/ml), dose2 (25mg/ml), dose3 (12.5mg/ml) and dose4 (6.25mg/ml). For the tests, 10 stage 3 larvae were placed in glass beakers in 20ml of water from the gites. Using a pipette, 0.5ml of each of the previously prepared doses was poured into the beaker, left at room temperature and the number of deaths determined at the following times: 24h, 48h of exposure.

At each concentration, the test is repeated 4 times. For each test, we use a control tube containing 10 larvae and 20 ml of gite water.

Dead larvae were considered to be those that were immobile and had fallen to the bottom of the beaker. The observation was made with the naked eye. The larval mortality rate was corrected using Abbott's formula (1925).

Statistical analysis of the means was carried out using the ANOVA analysis of variance test with Minitab version 19 software.

## II. Results and discussion

### II.1 Phytochemical screening

The results of the phytochemical tests are given in the table below.

**Table 1: Results of phytochemical screening.**

Parts of Plant	Extraction solvents	Polyphenols	Flavonoids	Tannins		Alkaloids
				Condensed	Hydrolysable	

Commented [A5]: ml

Commented [A6]: Harmonise the units of values

Commented [A7]: 50 mg/ml : there is a space between the number and the letter

Commented [A8]: Dose 2 ; dose 3

Commented [A9]: 0.5 ml

Commented [A10]: The table 1 is badly presented. You can refer to this journal article "Adsorption of Copper and Cadmium from Wastewater Using Chemically Activated Watermelon Peels as Adsorbent." Ukeme D. Archibong , Edidiog E. Ikpe and Osemudiamhen D. Amienghemhen Volume 33, Issue 1, Page 1-11, 2024; Article no.CSUI.102638. Or you can present it as your table 2

Leaves	Cyclohexane	-	-	+	-	-
	Chloroform	-	-	-	-	+
	Methanol	+++	+++	+++	+++	-

+++ : Strongly present; ++ : Moderately present; + : Weakly present; - : Absent.

**Commented [A11]:**

You have carried out qualitative tests and not quantitative ones. So you should simply put: +: present and -: absent.



**Figure 1:** Phytochemical testing equipment in the laboratory

**Commented [A12]:** Do not underline

From the results obtained in the table above, it should be noted that the methanolic extract of *Cassia siebérana* leaves is very rich in polyphenols, flavonoids and tannins (condensed and hydrolysable).

Condensed tannins are only slightly present in the cyclohexane extract and completely absent in the chloroform extract.

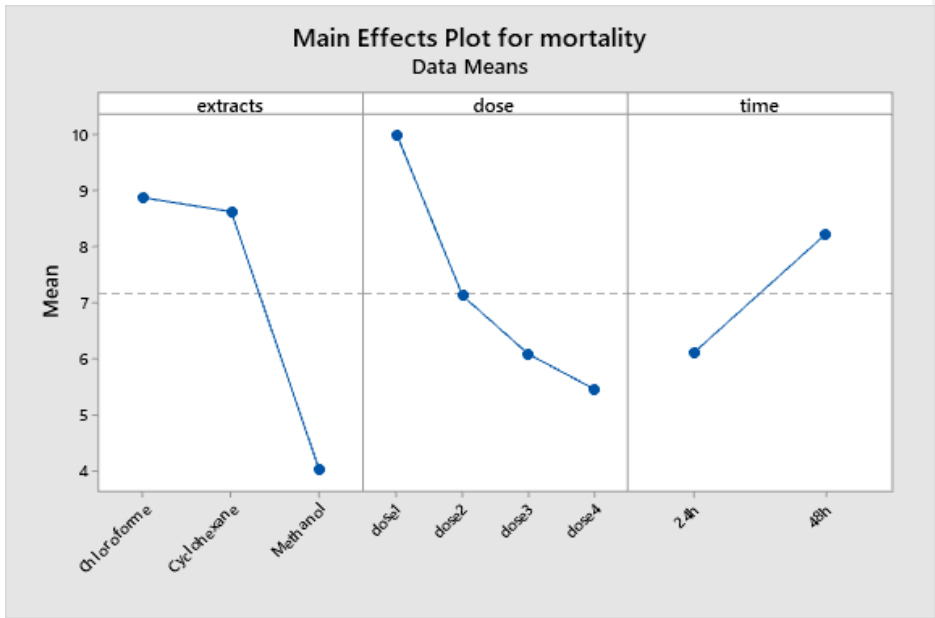
Alkaloids are also present in small quantities in the chloroform extract of the leaves.

The work of K. S. EVENAMEDE *et al.* [7] also showed that alcoholic extracts of leaves of the same species from Togo also contain flavonoids, polyphenols and tannins. However, alkaloids are absent in the Togo species. This can be explained by a difference in several parameters either geographical, physicochemical or biological such as: the difference in the harvesting site including the environment of the plant, the light, the season, but also and above all the harvesting period and the extraction procedure used [8].

The results obtained in this study are similar to those of Eliasse Zongo *et al.*, [9] whose work was recently carried out (January 2023) in the Hauts-Bassins region of Burkina Faso. They found virtually the same composition of secondary metabolites in the species harvested.

## II.2 Biological tests

The results of the larvicidal activity of the different extracts tested on *Anopheles gambiae* are presented in Figures 2 and 3 and Table 2.



**Figure 2:** Mortality curve as a function of extract, dose and time



**Figure 3:** Interaction between extracts and doses, extracts and time, doses and time.

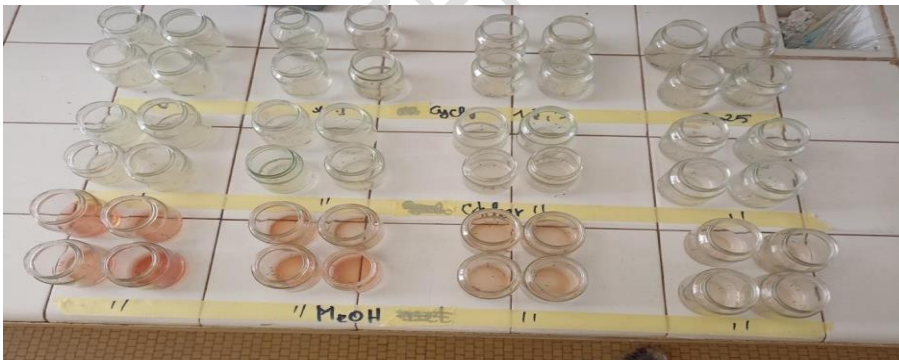
Commented [A13]: You don't underline

**Table 2:** Analysis of variance

Source	SomCar		SomCar CM		F value	
	DL	séq	Contribution	ajust		
extracts	2	482,33	41,75%	482,33	241,167	78,42
doses	3	290,92	25,18%	290,92	96,972	31,53
temp	1	108,38	9,38%	108,38	108,375	35,24
Error	89	273,71	23,69%	273,71	3,075	
Inadequate fit	17	243,71	21,09%	243,71	14,336	34,41
Pure error	72	30,00	2,60%	30,00	0,417	
Total	95	1155,33	100,00%			

Source	Value of p
extracts	0,000
doses	0,000
temp	0,000
Error	
Inadequate fit	0,000
Pure error	
Total	



**Figure 4:** Biological testing equipment in the laboratory

After exposing *Anopheles gambiae* larvae to different concentrations of the three extracts for 48 hours, the mortality rate varied according to the concentration. The higher the concentration, the greater the mortality.

The analysis of variance of the mean mortality of the larvae shows that there is a significant difference between the 4 doses of extracts used ( $P < 0.05$ ).

The results obtained show a variable sensitivity of the larvae, reflected in low to very high mortality rates when moving from one extract to another. The chloroform extract proved to be the most effective, with a mortality rate of just under 90% after 48 hours'

exposure. This activity could be explained by the presence of alkaloids in the chloroform extract, detected during phytochemical tests. Matasyoh *et al.* [10] showed a high larvicidal efficacy of alkaloids extracted from the leaves of *Zanthoxylum leprieurii*.

These results also show that larvicidal activity depends on the length of time the larvae are exposed to the different extracts. In fact, an increase in mortality was recorded as a function of exposure time.

Analysis of the interaction diagrams shows that all the extracts are equally sensitive to dose 1 (100% mortality after 48 hours' exposure). In the short term, the chloroform extract was slightly more effective than the alcoholic extract. However, in the long term, the opposite was observed.

The results obtained for the main effects of the extracts are perfectly consistent with the observations reported by Issoufou *et al.*, [11] who state in their study on *Phytochemical Characterization and Larvicidal Activity of Raw Plant Extracts from the Traditional Pharmacopoeia of Niger on Anopheles gambiae Larvae* that lethality increases as a function of the concentration and duration of exposure of the larvae.

Research conducted by Aziz Bouchelta [12] on the biocidal effects of alkaloids, saponins and flavonoids extracted from *Capsicum frutescens* L but also the work of Ndiaye. A [13] on phytochemical testing and larvicidal activity of three organic extracts of *Indigofera pilosa* stem on mosquito larvae revealed that alkaloid-containing extracts were among the most effective. These previous studies corroborate the results of the present study.

## Conclusion

Mosquitoes have always been considered a source of nuisance for humans, mainly because they can be vectors of disease.

In view of the problems associated with the use of chemical insecticides and their harmful impact on health and the environment, the use of natural alternatives that fulfil the same role and offer ecological advantages is necessary, hence the purpose of this study.

These results show good larvicidal activity of the three *Cassia siéberiana* leaf extracts. All the extracts tested constitute promising larvicides for the control of mosquitoes and by ricochet against malaria even if the lethality rate varies from one extract to another. In the future, we plan to fractionate and purify the most active extracts in order to isolate the molecule(s) responsible for the biological activity.

## COMPETING INTERESTS ?

## REFERENCES

- [1] WHO, "Treatment of uncomplicated *P. falciparum* malaria in the first trimester of pregnancy: Implementation of revised WHO guidelines (25 November 2022)," 2023.
- [2] Katy Heu, Mathilde Gendrin. Mosquito microbiota and its influence on vector transmission. *Biology Today*, 2018, 212 (3-4), pp.119-136. [ff10.1051/jbio/2019003](https://doi.org/10.1051/jbio/2019003). [ffpasteur-02919246](https://doi.org/10.1051/jbio/2019003).

Commented [A14]: to review?

Commented [A15]: to reformulate correctly or differently

Commented [A16]: ? to review

Commented [A17]: The bibliographic reference must be clearly stated.  
If you consulted the document on the Internet, you must specify the website and the date.

Commented [A18]: ?

- [3] Maifi Radhia, Salmi Meriem, " Study of the larvicidal activity of aqueous extract of *Ruta graveolens* against a species of mosquito *Culesita longiareoleta*," University of Larbi Tébessi -Tébessa, 2017.
- [4] J. MOUCHET; G. PICHON, P. GAYRAL & J. HAMO, "Sensitivity and resistance to insecticides of *Aedes aegypti* in West Africa and methods of controlling this vector," Bull. Org. world. Health, vol. 1971, No. % 145, pp. 394-404, 1963.
- [5] Victorin Houmènou, Arlette Adjatin, Fidèle Assogba, Joachim Gbénou, Akpovi Akoègninou, " Phytochemical and Cytotoxicity Study of Some Plants Used in the Treatment of Female Infertility in South Benin," European Scientific Journal, vol. 14, 2018.
- [6] WHO, Procedures for testing insecticide resistance in malaria vector mosquitoes, Second edition, 2018.
- [7] Evenamede, K. S., Kpegba, K., Simalou, O., Boyode, P., Agbonon, A., & Gbeassor, M., "Comparative study of antioxidant activities of ethanolic extracts of leaves, barks and roots of *Cassia sieberiana*," International Journal of Biologic, vol. 11, no. % 16, pp. 2924-2935, 2017.
- [8] Farnaz Malik, Shahzad Hussain, Alia Sadiq, Ghazala Parveen, Amina Wajid, Shazia Shafat, Rafique Ahmad Channa, Rashid Mahmood, Humayun Riaz, Muhammad Ismail, Fahadiya Yasin Raja, "Phyto-chemical analysis, anti-allergic and anti-inflammatory activity of *Mentha arvensis* in animals," African Journal of Pharmacy and Pharmacology, vol. 6, no. % 19, pp. 613-619, 2012.
- [9] Zongo E, Busuioic A, Meda RN-T, Botezatu AV, Mihaila MD, Mocanu A-M, Avramescu SM, Koama BK, Kam SE, Belem H, et al, "Exploration of the Antioxidant and Anti-inflammatory Potential of *Cassia sieberiana* DC and *Piliostigma thonningii* (Schumach.) Milne-Redh, Traditionally Used in the Treatment of Hepatitis in the Hauts-Bassins Region of Burkina Faso," Pharmaceuticals, vol. 16, no. % 11, p. 133, 2023; <https://doi.org/10.3390/ph16010133>.  
Malaria Vector *Anopheles gambiae*," The Natural Products Journal, vol. 1, no. % 12, pp. 121-124, 2011.
- [11] Issoufou Yolidjé, Djibo Alfa Keita, Idrissa Moussa, Abdoulaye Toumane, Inoussa Maman Maarouhi, Karim Saley, Jean-Luc Pirat, Tilman Much et Jean Maurille Ouamba, " Phytochemical Characterization and Larvicidal Activity of Raw Plant Extracts Iss of the Traditional Pharmacopoeia of Niger on the Larvae of *Anopheles gambiae* S.L.," European Scientific Journal, vol. 15, no. % 112, pp. 30-51, 2019.
- [12] Aziz Bouchelta, Ahmed Boughdad, Abelali Blenzar, "Biocidal effects of alkaloids, saponins and flavonoids extracted from *Capsicum frutescens* L. (Solanaceae) on *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae)," Biotechnol. Agron. Soc. Environ. 2005, vol. 9, n° % 14, p. 259-269, 2005.

**Commented [A19]:** For exemple : Ikpe EE, Esua E, Ekwere I, Willie I, Olumide A. Analytical assessment on the removal of phenol from aqueous solution using orange peel based activated – carbon. American Journal of Engineering Research. 2017;6(12):129–133.

**Commented [A20]:** The reference 10 is missing

- [13] Ndiaye, A., Diop, M. and Diouf., E. "Phytochemical test and larvicidal activity of three organic extracts from Indigofera pilosa stems on mosquito larvae," Open Access Library Journal, , vol. 4, pp. 1-8., 2017.

Take one article from the journal and look at how the references are quoted.

**Commented [A21]:** Harmonising bibliographical references and complying with the journal. Take one article from the journal and look at how the references are quoted.

UNDER PEER REVIEW